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Antibody-Mediated Protection of Mucosal Surfaces

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1 Introduction

Mucosal surfaces of the oral cavity, the digestive and urogenital tracts, and the airways are protected against environmental pathogens by innate and adaptive immune defense mechanisms. Innate defense involves physical, chemical and cellular factors. Entrapment of pathogens in mucus facilitates their clearance by peristalsis in the gut and ciliary movement in the airways. The longitudinal flow of fluids across the epithelial layer mediated by chloride channels helps to flush away microorganisms and prevent their attachment to the epithelial cell surface. The apical cell surface-associated glycocalyx, which consists of a dense network of

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glycoproteins, also limits the access of pathogens to the epithelial surface. Chemical factors (lysozyme, lactoferrin, peroxidase and defensins) secreted by specialized epithelial cells, such as the Paneth cells in the crypts of the small intestine, gastric acid and intestinal hydrolases (proteases, lipases, nucleases) constitute an efficient defense mechanism.

Host leukocytes present or recruited into mucosal tissues following microbial-epithelial cell interactions actively participate in innate defense. The recruited cells include monocytes, macrophages, neutrophils and eosinophils. These cells preferentially express Fc α receptors for immunoglobulin A (IgA) antibodies in both mice (HAYAMI et al. 1997) and humans (MONTEIRO et al. 1990) in mucosal tissues. These Fc α receptor-bearing cells may help to clear microorganisms, following opsonization by secretory IgA (sIgA) antibodies, facilitate uptake of antigen-antibody complexes by professional antigen-presenting cells and modulate immune functions by controlling the release of specific cytokines. Therefore innate immunity may not only provide rapid anti-microbial defense, but may also determine how pathogens activate the adaptive mucosal immune system and control the nature of the immune response (FEARON and LOCKSLEY 1996; BENDELAC and FEARON 1997). Adaptive immunity requires that the antigens cross the epithelium to reach the underlying organized lymphoid tissues where antigen presentation and priming of T and B lymphocytes can occur.

2 Humoral Immune Responses in Mucosal Tissues

sIgA antibodies play a major role in the protection of mucosal surfaces which is reflected by the very large quantity of antibodies produced and transported into secretions each day (~10g). Following stimulation by antigens, naive B cells in organized mucosal lymphoid tissues (MALT) of the gut, the airways and the oropharyngeal cavity move to the germinal center where they proliferate clonally. During clonal expansion, B cells undergo affinity maturation, first, by somatic hypermutation which generates variability in B-cell receptors and, second, by selection of those with highest affinity for the antigen. Selection of cells bearing these mutated receptors by the antigen occurs on the surface of the follicular dendritic cells a process which rescues cells expressing high affinity Ig receptors from apoptosis (for review, see LIU et al. 1992).

In MALT germinal centers, B lymphocytes undergo isotype switch and differentiate further into B cells that express IgA receptors. (CEBRA et al. 1991). MALT CD4⁺ T cells have been shown to promote IgA isotype switch of IgM-bearing B cells (KAWANISHI et al. 1983). Cytokines produced by activated Th2 helper CD4⁺ T cells, including IL-5, IL-10 and transforming growth factor beta (TGF- β) play a major role in triggering switch, but the precise molecular mechanism that mediates the recombination event has not yet been fully elucidated (STROBER and EHRHARDT 1994). Ligation of co-stimulatory molecules, such as

CD40, provides an important signal for switch induction (DEFrance et al. 1992). Bacterial lipopolysaccharide (LPS) has been shown to stimulate expression of the recombination machinery in pre-B lymphocytes (Li et al. 1996) and mucosal adjuvants, including cholera toxin and *E. coli* heat-labile toxin, are known to facilitate switch (LYCKE and STROBER 1989).

Subsequently, B lymphocytes differentiate into effector or memory cells, following contact with T helper lymphocytes and CD40-CD40 ligand interactions (LIU et al. 1991). In MALT, stimulated B and T cells acquire a mucosal homing program. The effector and memory lymphocytes lose their adhesion to stromal cells, leave organized MALT structures and enter the blood stream via the lymph. Depending on the mucosal sites at which priming takes place, different homing receptors will be expressed by B lymphocytes. Virtually all IgA- and even IgG-antibody-secreting cells detected after peroral and rectal immunization expressed $\alpha 4\beta 7$ integrin receptors, with only a minor fraction of these cells expressed the peripheral L-selectin receptor. In contrast, circulating B cells, induced by intranasal immunization, co-express L-selectin and $\alpha 4\beta 7$ receptors. This may explain the compartmentalization of mucosal immune responses initiated in the upper vs the lower aerodigestive tract (QUIDDING-JÄRRINK et al. 1997).

Effector and memory B cells are able to home to distant mucosal tissues or return to MALT structures (ROTT et al. 1997 and, for review, BUTCHER and PICKER 1996) (Fig. 1). The lymphocytes expressing mucosal $\alpha 4\beta 7$ homing receptors interact with flat post-capillary venule endothelial cells bearing mucosal addressins on their luminal surface (for review, see BUTCHER and PICKER 1996). Antigen receptors (surface Igs) do not participate in the selectivity of lymphocyte binding to the vascular bed. It has been suggested that antigen-specific plasmablasts become locally enriched in mucosal sites via retention at sites of antigen deposition (BUTCHER and PICKER 1996).

The mucosal addressin MadCam-1 (mucosal addressin cell adhesion molecule) is preferentially expressed in human and mouse intestinal flat post-capillary venules of the lamina propria and high endothelial venules of organized MALT, but not in other mucosal tissues (BRISKIN et al. 1997). MadCam-1, in mice, is also found associated with the endothelial cells of mammary-gland post-capillary venules. The vascular addressins mediating selective binding of lymphocytes in the airways and the genital tract have yet not been identified. Extravasation of lymphocytes requires the action of chemokines (for review, see BUTCHER and PICKER 1996). B lymphocytes express a specific chemokine receptor, which when knocked out prevents lymphocytes from reaching mucosal tissues (FÖRSTER et al. 1996). The chemokine that attracts B cells has recently been identified (GUNN et al. 1998; LEGLER et al. 1998), but it is not known whether B cell-specific chemokines are involved in the recruitment of B cells in mucosal tissues.

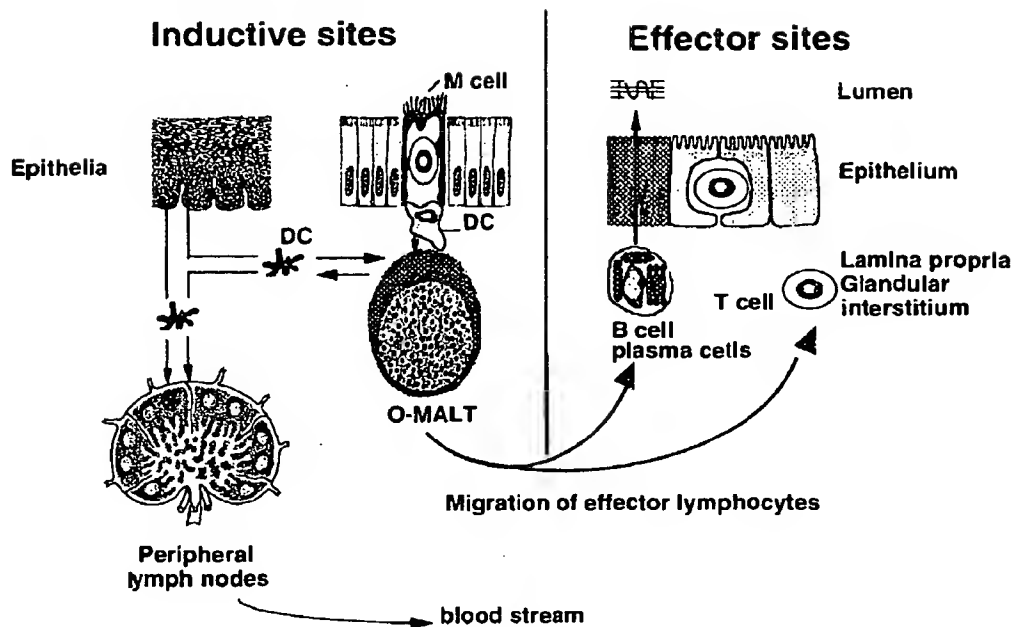


Fig. 1. In stratified epithelia (tonsil, vagina), antigen sampling is accomplished by migratory dendritic cells that transport antigens to distant lymph nodes or to local organized mucosa-associated lymphoid tissues (O-MALT). In simple epithelia (intestine, bronchi), membrane-associated (M) cells selectively take up the antigens and deliver them to underlying local O-MALT. Following antigen stimulation in lymphoid tissues, effector lymphocytes enter the submucosal lymphatics, enter the blood circulation and establish themselves in distant MALT and glandular sites. Migration to effector sites depends on the specific interaction between homing receptor on the lymphocytes and addressin on the vascular bed bathing the epithelia. B-cell differentiation into antibody-producing cells leads to the local production of immunoglobulin A (IgA) which, upon association with the polymeric immunoglobulin receptor (pIgR), are transported across the epithelial layer and serve to neutralize pathogenic agents.

3 Maturation of B Cells within Plasma Cells in Mucosal Tissues

Locally, in the lamina propria, effector B lymphocytes differentiate into antibody-secreting plasma cells, this process being regulated by T lymphocyte-, and epithelial, cell-derived cytokines (Fig. 2). In the intestinal mucosa, the number of plasma cells producing IgA exceeds those producing all other Ig isotypes. Maturation of IgA-bearing B cells into plasma cells is triggered by T cell-derived IL-5 (MATSUMOTO et al. 1989) and epithelial IL-6 (MCGHEE et al. 1991). In IL-6-deficient mice, a reduced number of IgA-producing plasma cells has been observed in the respiratory tract, and targeting IL-6 DNA into bronchial epithelial cells restored maturation of IgA B cells into plasma cells (RAMSAY et al. 1994). This result, however, has not been confirmed within the digestive tract, suggesting that IL-6 is probably not the only cytokine involved in B cell maturation (BROMANDER et al. 1996). In the mucosal environment, all plasma cells, irrespective of their Ig isotype, express J chain, the small polypeptide required for IgA polymerization (BJERKE and BRANDTZAEG 1990).

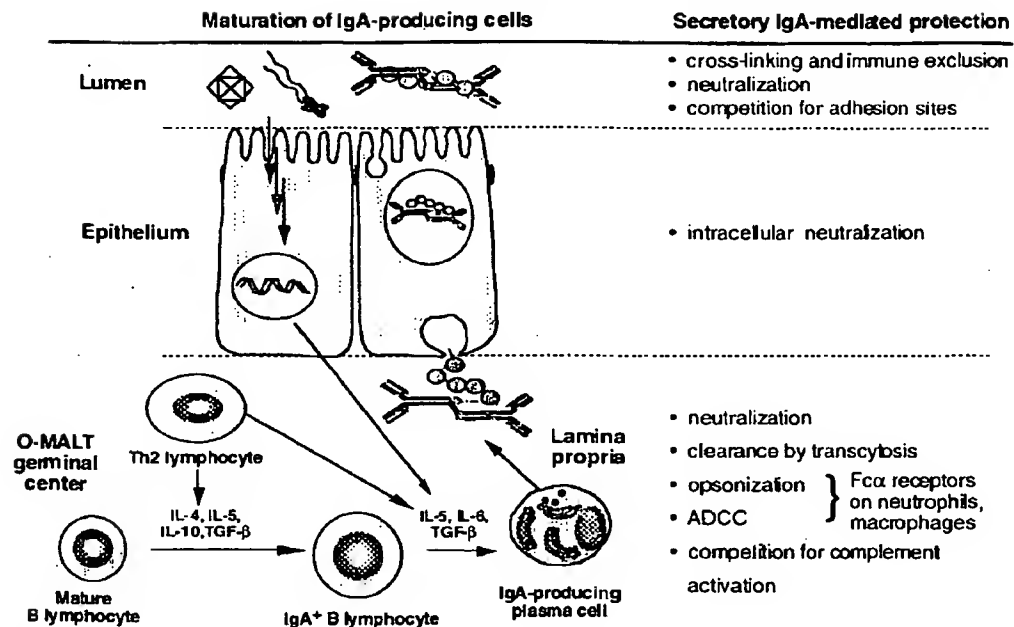


Fig. 2. Role of cytokines in the immunoglobulin A (IgA) response and biosynthesis. Mature (antigen responsive) B lymphocytes expressing membrane IgM switches to surface IgA production, under the control of Th2 cytokines, including transforming growth factor beta (TGF- β), interleukin (IL)-4, IL-5 and IL-10. This happens most likely in the germinal centers of organized mucosa-associated lymphoid tissues (O-MALT). IgA⁺ cells eventually leave the O-MALT and migrate to distant mucosal and glandular sites to undergo final differentiation. Enterocyte-derived IL-6 and Th2-released IL-5 and TGF- β induce secretion of soluble IgA oligomers through concomitant induction of J chain expression. Following binding to polymeric immunoglobulin receptor (pIgR), J chain-containing IgA polymers are selectively transported across the epithelium by transcytotic vesicles which, following fusion with the apical membrane, release sIgA complexes comprising IgA and the clipped form of pIgR called secretory component (SC). The possible sites of action of IgA/sIgA are listed on the right and discussed case by case within the text.

4 Transepithelial Transport of Mucosal Immunoglobulins

The prevention of microbial infection requires that the humoral immune effectors gain access to the luminal compartment to block adhesion and invasion of environmental pathogens. Simple epithelia are sealed at their apex by tight junctions that prevent the lateral diffusion of antibodies between cells. Thus, translocation of antibodies from their site of synthesis in the lamina propria to the luminal compartments necessitates an efficient transepithelial transport machinery (Fig. 2). The pathway followed by polymeric Igs (IgA and IgM) across epithelial cells has been extensively studied (SOLARI and KRAEHNBUHL 1984; MOSTOV and DEITCHER 1986; BARROSO and SZTUL 1994; NATVIG et al. 1997; WEIMBS et al. 1997), and the receptor that mediates transcytosis has been well characterized (EIFFERT et al. 1984; KRAJCI et al. 1989; SCHAEFER et al. 1991; COYNE et al. 1994).

The polymeric immunoglobulin receptor (pIgR) is expressed at the basolateral surface of a variety of epithelial cells, including small and large intestinal, nasal, tracheal and bronchial, and cervical and uterine epithelial cells of all mammals studied so far (BRANDTZAEG 1994). The receptor comprises five extracellular domains of 110–120 residues which share homology with V κ and V H Ig domains, a 23-amino acid long membrane-spanning segment, and a cytoplasmic tail of about 100 amino acids (MOSTOV et al. 1984). Human, rat, mouse, rabbit and bovine receptors show extensive homology, particularly in the first domain and in the cytoplasmic tail. Following polymeric IgA binding and internalization into the recycling basolateral endosomal compartment, the complex is sorted into transcytotic vesicles that reach the apical endosomal compartment (SONG et al. 1994), where the ligand–receptor complex recycles transiently (APODACA et al. 1994).

The presence of the J chain in polymeric IgA accounts for the selective discrimination of polymeric vs monomeric IgA (BRANDTZAEG and PRYDZ 1984). At the apical surface, the poly-Ig receptor is proteolytically cleaved, and the extracellular fragment known as secretory component (SC) is released, whether bound or not to its polymeric IgA ligand. Rabbits express a spliced version of the poly-Ig receptor lacking domains 2 and 3 (DEITCHER and MOSTOV 1986) which is fully competent for transport (SOLARI and KRAEHNBUHL 1987) and generates SC non-covalently bound to IgA (FRUTIGER et al. 1987). The information necessary for the complex trafficking of the pIgR in epithelial cells resides within its cytoplasmic tail. The sequences that control basolateral sorting and endocytosis (MOSTOV et al. 1992), which are highly conserved among species (BANTING et al. 1989), are flanked by serine residues that undergo phosphorylation once the receptor is terminally glycosylated in the Golgi apparatus (HIRT et al. 1993). Transcytosis of the poly-Ig receptor is stimulated by binding of polymeric IgA (SONG et al. 1994). Endocytosis and transcytosis are regulated by the trimeric G proteins, protein kinase C and calmodulin (CHAPIN et al. 1996). In rat liver, the asialoglycoprotein receptor (ASGP-R) has also been shown to participate in IgA endocytosis (SCHIFF et al. 1986). In humans, transport of IgA from blood to bile is minimal, due to the lack of pIgRs in hepatocytes.

IgG antibodies are also found in secretions and it has been proposed that they cross the epithelial barrier by transudation. Recently, however, it has been shown that small-intestinal enterocytes express the neonatal Fc receptor (ISRAEL et al. 1997). In newborn rodents, the receptor mediates transport of maternal milk IgG antibodies from the lumen to the lamina propria. Binding of IgG antibodies in the luminal compartment is facilitated by low pH and release in the interstitium by neutral pH (RODEWALD and KRAEHNBUHL 1984). Transport in the opposite direction requires that IgG antibodies are able to reach an acidic compartment in order to bind to Fc receptors and escape to the lysosomal pathway. In the yolk sac, IgGs are taken up by fluid-phase endocytosis and accumulate in an acidic endosomal compartment where binding to neonatal Fc receptors takes place (ROBERTS et al. 1990). Whether the neonatal Fc-receptor mediates IgG transudation in other mucosal epithelia remains to be established.

5 Antibodies in Mucosal and Glandular Secretions

In human, roughly 60% of all Igs produced are IgAs, of which about half are selectively transported into external secretions (MESTECKY and MCGHEE 1987; KERR 1990). The majority of sIgA in humans is derived from local synthesis, and not from the circulation. Humans have two subclasses of monomeric and polymeric IgA: IgA1 and IgA2 (KAWAMURA et al. 1992), which differ in their hinge region and in their carbohydrate composition (WOLD et al. 1995). IgA1, but not IgA2, can be cleaved by IgA-specific proteases produced by a number of bacteria (PLAUT 1988). The protease recognizes a distinct sequence in the hinge region of IgA1 antibodies and renders the antibodies less efficient in cross-linking microorganisms. SC does not protect against cleavage (PLAUT et al. 1985). The proportion of IgA1 and IgA2 varies in individual secretions. IgA1-producing cells predominate in most mucosal tissues and glands, including the tonsils, the stomach, the duodenum, the mammary gland and the respiratory tract, while IgA2-secreting cells populate, preferentially, the large intestine and the female genital tract. Antibodies specific for protein antigens are found predominantly in the IgA1 subclass, whereas bacterial LPS and carbohydrate antigens lead preferentially to IgA2 antibody production. In IgA-deficient individuals and animals, a compensatory increase of sIgM in secretions has been reported (PLEBANI et al. 1983), and experimental IgA-deficient mice challenged with *H. felis* and cholera toxin have much higher titers of IgM antibodies than wild-type animals (NEDRUD et al. 1996).

IgE are also locally produced by plasma cells. They play a major role in the lamina propria where they can interact and activate local mast cells. IgE can mediate protection against certain parasites, as demonstrated in response to the feeding of larval ticks (MATSUDA et al. 1990). Recruitment of leukocytes at sites of IgE-dependent immune response by tumor necrosis factor alpha (TNF- α) released by mast cells could be a critical component of natural immunity to infection of bacteria and other pathogens. It has been reported that not only Th2 cells, but also mast cells and the B cells that mediate IgE responses express the same chemokine receptor, the eotaxin receptor CCR3 (SALLUSTO et al. 1997), which facilitates their recruitment at mucosal sites. Due to their strategic anatomical distribution near surfaces exposed to the external environment and their ability to release mediators of inflammation, mast cells are well suited to function as sentinels of mucosal immunity.

5.1 Molecular Structure of Immunoglobulin A (IgA)

Secretory sIgA has unique features that make this antibody particularly well suited to protect mucosal surfaces. Its polymeric structure enhances its avidity for antigens and pathogens, its distinct carbohydrate composition contributes to its

binding to mucus components and also to its resistance to proteolysis (KERR 1990; MESTECKY et al. 1991). Secretory IgA consists of at least two monomeric IgA units and two additional polypeptide chains, a J chain and SC (Fig. 3). These four polypeptides are produced by two distinct cell types. The Ig heavy and the light chains and the J chain are synthesized and assembled by plasma cells, while SC, which corresponds to the five extracellular domains of the poly-Ig receptor, is contributed to by epithelial cells of mucus membranes and exocrine glands.

5.1.1 Polypeptide Composition of Secretory (s) IgA

Mammalian Ig α heavy chain consists of three constant-region domains (C α 1–C α 3), composed of seven β strands in a four–three configuration with intervening loops to create a β -barrel conformation (HUNKAPILLER and HOOD 1989). In humans, non-human primates and rabbits, multiple α -chain isotypes have been described (SPIEKER-POLET et al. 1993), in contrast to all other species, where only one IgA isotype is found. In human, two subclasses of IgA have been described: IgA1 and IgA2. The major difference between these two subclasses lies in the hinge region, lacking a 13-amino acid stretch in the IgA2 molecule. In addition, the IgA2

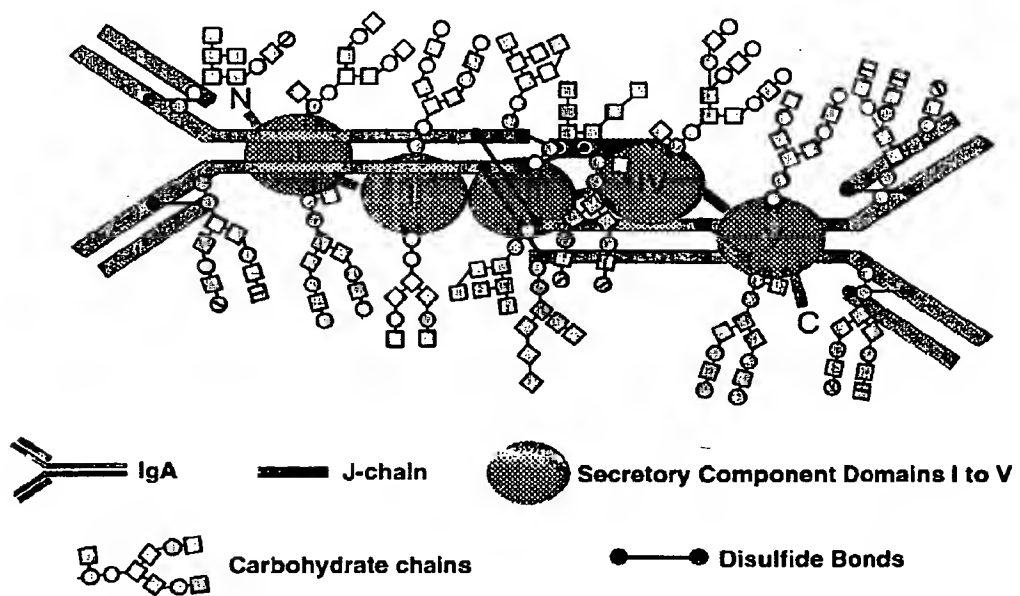


Fig. 3. Schematic representation of a dimeric secretory immunoglobulin A (sIgA). Two IgA monomers are depicted in a tail-to-tail arrangement, with J chain covalently linked to one monomer through two disulfide bridges. Secretory component (SC) is made of five Ig-like domains and corresponds to the extracellular portion of polymeric immunoglobulin receptor (pIgR). Domain I contains the information for initial anchoring to oligomeric IgA, while two cysteine residues situated in the IgA α chain and the domain 5 of SC form a covalent bond in several species. Sugar moieties most likely contributing to the stability and solubility of sIgA are also drawn, yet not on scale

subclass comprises two allotypes: IgA2 m(1) and IgA2 m(2), the major structural differences of which lie in the arrangement of the α and light chain interchain disulfide bridges. The C α 3 domain displays, at its C terminus, an 18-amino acid tail that, during biosynthesis of IgA, associates with J chain.

J chain is a 15-kDa glycoprotein, covalently linked to polymeric IgA or IgM before secretion by plasma cells. The role of J chain in the polymerization process remains controversial (CATTANEO and NEUBERGER 1987; DAVIS et al. 1989). IgA dimer formation occurs in J chain-deficient mice, but the IgA dimer/monomer ratio in serum is perturbed (HENDRICKSON et al. 1995); hepatic IgA transport is impaired, whereas intestinal, breast milk and nasal IgA levels compare with wild-type animals (HENDRICKSON et al. 1996). J chain-deficient IgA found in secretions, however, is not associated with SC. These data confirm the essential role of J chain in interaction with the poly-Ig receptor (BRANDTZAEG and PRYDZ 1984).

SC represents the extracellular portion of the poly-Ig receptor. The three-dimensional structure of SC has not been elucidated, but molecular modeling of rabbit domains 1 and 2 indicates that both domains consist of the typical nine Ig β strands (A, B, C, C', C, D, E, F, G) connected by loops of variable size (COYNE et al. 1994; CORTHESEY et al. 1996). Each domain contains an internal disulfide bond, characteristic of Ig homology units, which apparently links strand B with strand F. In human sIgA, amino acids 14–38 in the N-terminus domain 1 of SC constitute the IgA binding epitope (BAKOS et al. 1991). Cysteine 467 in the distal fifth domain participates in disulfide bridge formation with cysteine 311 of one C α 2 domain of dimeric IgA. It is not known in which cell organelle covalent disulfide linkages are established during transcytosis, but the process is rapid and spontaneous, as reflected in *in vitro* reconstitution experiments (RINDISBACHER et al. 1995). SC delays IgA degradation by intestinal proteases, but does not alter the affinity for the antigen (LÜLLAU et al. 1996).

5.1.2 Glycosylation of sIgA

The glycosylation pattern of sIgA is complex and highly heterogeneous (ENDO et al. 1994; WOLD et al. 1994). The 13-amino acid stretch in the IgA1 hinge region contains five O-linked glycans, recognized by the lectin Jacalin (ROQUE-BARREIRA and CAMPOS-NETO 1985), which presumably enhances the flexibility between Fc and F(ab')₂ fragments. The presence of this extra sequence in IgA1 molecules is responsible for the sensitivity of the antibody to bacterial specific proteases as discussed above. Glycosylation of the α chain is essential for intracellular stability and normal secretion of IgA (TAYLOR and WALL 1988). In contrast, absence of glycosylation does not lead to loss of antigen binding activity (DONADEL et al. 1994). Human SC purified from milk is heavily glycosylated with 5–7 N-linked sugar side chains, accounting for over 20% of its molecular mass (MIZOGUCHI et al. 1982). IL-4 and IL-5 have recently been shown to alter the terminal glycosylation pattern of IgA (CHINTALACHARUVU and EMANCIPATOR 1997). These authors suggest that increased production of IL-4 and IL-5 by peripheral blood lymphocytes from IgA nephropathy patients might result in the production of abnormally

glycosylated IgA which, in turn, may promote the disease by deposition of IgA in glomeruli.

5.1.3 Regulation of SC and α Chain Gene Expression

Poly-Ig receptor expression in mucosal epithelia is regulated by a complex interplay among lymphocytes, macrophages and epithelial cells. The presence of cytokine regulatory elements in the promoter of the poly-Ig receptor genes (PISKURICH et al. 1997) allows expression of the receptor to be upregulated in response to both Th1 and Th2 cytokines. Upregulation of poly-Ig receptor expression stimulates transcytosis of IgA antibodies. Several interferon-stimulated response elements (ISRE) have been identified in the human poly-Ig receptor promoter and first exon (PISKURICH et al. 1997; VERRIJDT et al. 1997). In HT-29 human colon carcinoma cells, poly-Ig receptor is upregulated by interferon gamma (IFN- γ) (PISKURICH et al. 1993), TNF- α (KVALE et al. 1988), and IL-4 (PHILLIPS et al. 1990). In contrast to the promoter ISREs, the ISRE in the first exon binds interferon responsive factor (IRF)-1, a member of the interferon regulatory-factor family, only after stimulation of HT-29 cells with IFN- γ . Many microorganisms are able to stimulate cytokine or chemokine secretion directly by epithelial cells or indirectly via intraepithelial lymphocytes or inflammatory cells recruited into mucosal tissues (ISHIKAWA et al. 1993; QUIDING et al. 1991). Upregulation of poly-Ig receptor by cytokines involves a protein tyrosine kinase-dependent signaling pathway (DENNING 1996), in which the cytokine receptors recruit cytosolic tyrosine kinases (Janus kinases or JAKs) which, in turn, activate transcription factors of the STAT (signal transduction and activation of transcription) family (IHLE et al. 1994).

Several enhancer regions (DARIAVACH et al. 1991; MATTHIAS and BALTIMORE 1993) and hypersensitive sites (MADISEN and GROUDINE 1994) identified downstream of the murine C α gene are involved in its developmental and transcriptional regulation. The best characterized enhancer element, 3' α E, contains motifs (octamer, κ B, G-rich sequence) that mediate upregulation of transcription during maturation to plasma cells (SINGH and BIRSSTEIN 1993; MEYER et al. 1995) as well as motifs (Elf-1, AP-1) for activation induced by B cell receptor cross-linking (GRANT et al. 1995). 3' α E also comprises the hypersensitive sites HS1 and HS2, which share 90% identity with their human α 1 and α 2 gene counterparts (MILLS et al. 1997). The HS1,2 core homology is likely to contain essential motifs important for the strong late B cell-specific enhancer characteristics of HS1,2 in mice and humans. Although the function of transcription binding sites in the human HS1,2 has not yet been demonstrated experimentally, this sequence element comprises Oct, AP-1, Ets, μ E5 motifs (MILLS et al. 1997), all of which are nearly identical to, and functional in, the murine HS1,2 enhancer.

5.2 Functions of sIgA Antibodies

It is usually accepted that sIgA in secretions bathing the mucosal surfaces protect these surfaces against environmental pathogens by cross-linking microorganisms or macromolecules, thus facilitating their elimination by peristalsis or mucociliary movement and preventing their contact with the surface of epithelial cells, a phenomenon called immune exclusion. The molecular mechanisms underlying sIgA antibody-mediated protection still remain poorly understood, and it is likely that this unique effector molecule plays multiple roles in addition to immune exclusion (Fig. 2).

The ability of secretory antibodies to recognize intact bacteria, viruses or parasites at mucosal surfaces is a prerequisite for protection. This is reflected by the observation that polyclonal sIgA responses to mucosal pathogens is dominated by antibodies recognizing microbial surface antigens. An antibody response is not restricted to pathogens, but commensals such as gram-negative microorganisms introduced into the gut of germ-free mice also produce an immune response (SHROFF et al. 1995). This suggests that a successful sIgA response can attenuate chronic stimulation of germinal-center reactions in gut-associated lymphoid tissue in response to bacteria persisting in the gut. The role of sIgA in the establishment and maintenance of the gut flora will require further investigation.

It has been difficult to evaluate the relative importance of sIgA in protection or to assess whether "immune exclusion" by sIgA alone is able to prevent mucosal infection. Techniques for the production of monomeric IgA antibodies have been developed by several groups during the last few decades (STYLES et al. 1984; COLWELL et al. 1986; RITS et al. 1986; MAZANEC et al. 1987; WELTZIN et al. 1989), and production of recombinant sIgA has been achieved in mammalian cells (CHINTALACHARUVU and MORRISON 1997; BERDOZ et al. 1998) and in plants (MA et al. 1995). This has allowed assessment of protection in vivo either by passive transfer of monoclonal antibodies specific for viruses (MAZANEC et al. 1987; RENEGAR and SMALL 1991), bacteria (MICHETTI et al. 1992) or toxins (APTER et al. 1991; APTER et al. 1993b). The implantation of IgA-producing hybridoma cells into the backs of mice has also allowed efficient delivery of antibodies into mucosal sites (WINNER et al. 1991). The development of methods (CZERKINSKY et al. 1983; HANEBERG et al. 1994) to measure antibodies in mucosal tissues has also contributed to a better understanding of their role in protection.

5.2.1 Immune Exclusion by Antigen Cross-Linking

Resistance to mucosal infection has been correlated with specific sIgA. The secretory antibodies provide an immunological barrier that prevents foreign antigens, including bacteria, viruses, parasites and toxins, from attaching to mucosal surfaces (for review see NEUTRA et al. 1991). Due to their multivalency, sIgA antibodies are ideally designed to cross-link target macromolecules or microorganisms in the mucosal environment. They do so by preventing their diffusion through the glycocalyx at the surface of the epithelial cells, by blocking their binding to epithelial

surface receptors, by inhibiting their motility or by facilitating their entrapment in mucus. Prevention of viral attachment and subsequent internalization represents a major mechanism by which IgA-coated viruses are neutralized (TAYLOR and DIMMOCK 1985). In the absence of mucus and clearance mechanisms, sIgA antibodies are able to protect epithelial cell monolayers (MICHETTI et al. 1994). Aggregation of the pathogens, however, requires antibody concentrations which probably cannot be reached in the mucosal environment. This suggests that protection mediated by sIgA must involve other mechanisms.

5.2.2 Interaction with Mucus

Mucus is generally thought to protect epithelial cells by forming a diffusional barrier with pore size of about 100 nm, through which only small molecules including antibodies can pass. IgG, IgG fragments, IgA and IgM diffuse as rapidly in cervical mucus as in water, and it has been proposed that particles as large as viruses can similarly diffuse through human cervical mucus, provided that the particle forms no adhesive interactions with mucus glycoproteins (SALTZMAN et al. 1994). Cross-linking of viral particles by sIgA antibodies, however, significantly impedes their diffusion in cervical mucus. Whether mucus from other mucosal surfaces behave similarly remains to be established. Coating hydrophobic bacteria such as *Salmonella typhimurium* or *Escherichia coli* with sIgA renders their surface more hydrophilic, thus facilitating their entry and movement into mucus gels (MAGNUSSON and STJERNSTROEM 1982; MCCORMICK et al. 1988). Thus, microorganisms coated with sIgA antibodies would be more readily retained in the moving stream of mucus. Secretory antibodies would bind to mucus only when tightly packed on the surface of the microorganisms which increases the avidity of the low affinity IgA-mucin interaction.

5.2.3 Neutralization and Opsonization

Most IgA antibodies do not neutralize microorganisms by standard mechanisms used by IgG. Generally, they do not opsonize via the macrophage Fc γ receptor in vitro, they do not activate the classical complement pathway and, thus, they do not lyse bacteria (KILIAN et al. 1988). There is evidence that sIgA can directly block the microbial sites that mediate epithelial attachment, either by binding to their adhesins or by sterically hindering their interaction with the epithelial cell surface (WILLIAMS and GIBBONS 1972; SVANBORG-EDEN and SVENNERHOLM 1978; WELTZIN et al. 1996). IgA antibodies which protect mucosal surfaces were shown to be inefficient when injected systemically (MICHETTI et al. 1992), suggesting that neutralization in a mucosal environment operates through different mechanisms than systemically (NEUTRA et al. 1994).

Several studies have demonstrated that protection of epithelial surfaces against viruses, bacteria or toxins can be accomplished with monoclonal IgA antibodies directed against microbial surface epitopes not necessarily involved in pathogenesis or adhesion (APTER et al. 1993a; PHALIPON et al. 1995).

Secretory IgA antibodies are able to cooperate with the innate defense system and enhance, for instance, the bacteriostatic activity of lactoperoxidase or lactoferrin present in mucosal fluids (TENOVUO et al. 1982). Inflammatory cells (neutrophils, eosinophils and macrophages) and immune cells (lymphocytes and monocytes) express Fc α receptors in mucosal tissues (FANGER et al. 1983; MONTEIRO et al. 1990). Occupancy and aggregation of the Fc α receptor on monocytes release pro-inflammatory cytokines (PATRY et al. 1995). The role of IgA-dependent cellular cytotoxicity which has been reported for the clearance of *Salmonella typhi* in mice (TAGLIABUE et al. 1985) has to be further investigated. It is likely that cell-mediated phenomena play an important role in protection of the host against mucosal and systemic infection once the pathogen has crossed the epithelial barrier and entered mucosal tissues.

5.2.4 Intracellular Neutralization and Antigen Clearance

Microorganisms that are internalized by epithelial cells in mucosal tissues and accumulate in endosomes could be neutralized intracellularly in endosomal compartments provided that there is a meeting point between virus-containing endosomes and IgA-containing endosomal vesicles. This has been tested experimentally: IgA monoclonal antibodies against Sendai virus, a parainfluenza virus, co-localized with the viral hemagglutinin-neuraminidase protein within infected epithelial cells and reduced intracellular viral titers (MAZANEC et al. 1995).

A mouse model and "backpack tumor" transplantation (WINNER et al. 1991) were recently used to determine the protective effect of antibodies against rotavirus capsid proteins. Two non-neutralizing IgA antibodies to VP6 were capable of preventing virus shedding in infected mice (BURNS et al. 1996). These antibodies were not active when administered to the luminal side of the intestinal tract. The lack of virus shedding in the stools was taken as an indication that virus infection could be prevented inside the infected cells. However, the decrease of virus shedding could result from inhibition of virus assembly. It is difficult to imagine how VP6 specific antibodies that do not have access to surface-exposed epitopes on the virus could prevent infection. In addition, rotavirus infects villus epithelial cells, particularly those on the upper villus and IgA antibodies are transported mainly in crypt cells. Thus, it is not clear how IgA antibodies can neutralize the virus intracellularly. When prevention of diarrhea was assessed, rather than virus shedding, anti-VP6 antibodies were unable to prevent diarrhea in newborn mice (RUGGERI et al. 1998).

Soluble dimeric IgA-antigen immune complexes are transported from the basolateral to the apical surface of poly-Ig receptor-expressing epithelial monolayers and released into the apical compartment (KAETZEL et al. 1991; KAETZEL et al. 1994). This indicates that the pathways of epithelial transcytosis of free and complexed dimeric IgA are the same. Given the high population density of mucosal IgA plasma cells and the enormous surface area of poly-Ig receptor-expressing mucosal epithelium, it is likely that significant local transcytosis of IgA immune complexes occurs in vivo, providing a means to eliminate microorganisms that have

gained access to mucosal tissues. Such a mechanism may thus provide an important defense function for IgA.

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